CLAIMS

We claim:

1. A method of purifying a troponin-tagged molecule, said method comprising contacting the troponin-tagged molecule with an affinity matrix that comprises a cognate ligand of the troponin tag, thereby immobilizing the troponin-tagged molecule on the affinity matrix.

2. A method according to claim 1, wherein said troponin-tagged molecule is contacted with said affinity matrix in the presence of calcium.

3. A method according to claim 2, wherein the affinity of binding between the troponin tag and said cognate ligand comprises a K_d greater than 10 nM.

4. A method according to claim 2, wherein said troponin-tagged molecule is released from said affinity matrix by adding an agent that chelates calcium.

5. A method according to claim 4, wherein said agent that chelates calcium is selected from the group consisting of EDTA, EGTA, BAPTA, citrate, and phosphate.

6. A method according to claim 1, wherein said troponin-tagged molecule is a fusion protein that comprises a troponin molecule and a polypeptide that is not a troponin molecule.

7. A method according to claim 6, wherein said fusion protein is produced recombinantly.

8. A method according to claim 6, wherein said fusion protein comprises at least one troponin molecule at the N-terminus.

9. A method according to claim 6, wherein said fusion protein comprises at least one troponin molecule at the C-terminus.

10. A method according to claim 6, wherein said fusion protein comprises at least one troponin molecule at an amino acid residue between the N-terminus and the C-terminus.

11. A method according to claim 6, wherein said fusion protein comprises a linker between said troponin molecule and said polypeptide that is not a troponin molecule.

12. A method according to claim 11, wherein said linker is a polypeptide.

13. A method according to claim 12, wherein said polypeptide linker comprises a protease recognition site.

14. A method according to claim 13, wherein said polypeptide that is not a troponin molecule is released from said affinity matrix by protease cleavage at the protease recognition site.

15. A method according to claim 1, wherein said troponin-tagged molecule comprises at least one molecule of troponin C, or a fragment or analogue thereof that is capable of specifically binding said cognate ligand on said affinity matrix.

16. A method according to claim 15, wherein said affinity matrix comprises a troponin C binding peptide.

17. A method according to claim 16, wherein said troponin C binding peptide comprises the sequence SRLDYLKSSLLHLGSR (SEQ ID NO:1), or a fragment or analogue thereof that is capable of specifically binding troponin C.

18. A method according to claim 17, wherein said troponin C binding peptide further comprises a cysteine residue at the N-terminus, and said affinity matrix is formed by reacting the troponin C binding peptide with a thiol reactive matrix.

19. A method according to claim 16, wherein the affinity matrix comprises a substrate selected from the group consisting of cross-linked polysaccharide, agarose, ceramic, metal, glass, plastic, and cellulose.

20. A method according to claim 16, wherein said troponin C-tagged molecule is released from said affinity matrix by adding a releasing agent, and wherein said releasing agent comprises a troponin C binding peptide.

21. A method according to claim 15, wherein said affinity matrix comprises troponin I, or a fragment or analogue thereof that is capable of specifically binding troponin C.

22. A method according to claim 21, wherein said troponin I is mutated to eliminate internal cysteine residues or to replace internal cysteine residues with other amino acids, wherein said troponin I comprises the sequence CCCSSSSSSSS (SEQ ID NO:3) at the N-terminus or the sequence SSSSSSSSCCC (SEQ ID NO:4) at the C-terminus, and wherein said affinity matrix is formed by reacting said troponin I with a thiol reactive matrix.

23. A method according to claim 22, wherein said internal cysteine residues are replaced with amino acids selected from the group consisting of serine and threonine.

24. A method according to claim 21, wherein the affinity matrix comprises a substrate selected from the group consisting of cross-linked polysaccharide, agarose, ceramic, metal, glass, plastic, and cellulose.

25. A method according to claim 21, wherein said troponin C-tagged molecule is released from said affinity matrix by adding a releasing agent, and wherein said releasing agent comprises troponin I, or a fragment or analogue thereof that is capable of specifically binding troponin C.

26. A method according to claim 21, wherein at least part of said method is performed in the presence of a denaturing agent.

27. A method according to claim 26, wherein said denaturing agent comprises urea.

28. A method according to claim 1, wherein said affinity matrix comprises troponin C, or a fragment or analogue thereof that is capable of binding the troponin tag on said troponin-tagged molecule.

29. A method according to claim 28, wherein said troponin-tagged molecule comprises at least one molecule of a troponin C binding peptide.

30. A method according to claim 29, wherein said troponin C binding peptide comprises the sequence SRLDYLKSSLLHLGSR (SEQ ID NO:1), or a fragment or analogue thereof that is capable of specifically binding troponin C.

31. A method according to claim 28, wherein said troponin C binding peptide-tagged molecule is released from said affinity matrix by adding a releasing agent, and wherein said releasing agent comprises troponin C, or a fragment or analogue thereof that is capable of specifically binding said troponin C binding peptide.

32. A method according to claim 28, wherein said troponin-tagged molecule comprises at least one molecule of troponin I, or a fragment or analogue thereof that is capable of specifically binding troponin C.

33. A method according to claim 32, wherein said troponin I-tagged molecule is released from said affinity matrix by adding a releasing agent, and wherein said releasing agent comprises troponin C, or a fragment or analogue thereof that is capable of specifically binding said troponin I.

34. A method according to claim 32, wherein at least part of said method is performed in the presence of a denaturing agent.

35. A method according to claim 34, wherein said denaturing agent comprises urea.

36. A method according to claim 28, wherein said affinity matrix is formed by reacting troponin C with a cyanogen bromide or glyoxal activated matrix.

37. A method according to claim 28, wherein said affinity matrix is formed by irreversibly linking troponin C to a substrate via reactive amino groups of said troponin C.

38. A method according to claim 28, wherein the affinity matrix comprises a substrate selected from the group consisting of cross-linked polysaccharide, agarose, ceramic, metal, glass, plastic, and cellulose.

39. A molecule purified according to the method of claim 1.

40. A molecule according to claim 39, wherein said molecule is a troponin-tagged fusion protein.

41. A method for detecting a troponin-tagged molecule according to claim 39, comprising contacting said troponin-tagged molecule with an antibody that specifically recognizes the troponin tag.

42. An affinity matrix comprising troponin C, or a fragment or analogue thereof that is capable of specifically binding to a troponin C binding peptide or troponin I, wherein said troponin C is attached to a substrate.

43. An affinity matrix according to claim 42, further comprising a bound troponin C binding peptide- or troponin I-tagged molecule.

44. A method of producing an affinity matrix according to claim 42, comprising reacting said troponin C with a cyanogen bromide or glyoxal activated matrix.

45. A method of producing an affinity matrix according to claim 42, comprising irreversibly linking said troponin C to a substrate via reactive amino groups of said troponin C.

46. An affinity matrix comprising a troponin C binding peptide attached to a substrate.

47. An affinity matrix according to claim 46, wherein said troponin C binding peptide comprises the sequence SRLDYLKSSLLHLGSR (SEQ ID NO:1), or a fragment or analogue thereof that is capable of specifically binding to troponin C.

48. An affinity matrix according to claim 46, further comprising a bound troponin C-tagged molecule.

49. A method of producing an affinity matrix according to claim 47, wherein said troponin C binding peptide further comprises a cysteine residue at the N-terminus, and said method comprises reacting the troponin C binding peptide with a thiol reactive matrix.

50. An affinity matrix comprising troponin I, or a fragment or analogue thereof that is capable of specifically binding to troponin C, wherein said troponin I is attached to a substrate.

51. An affinity matrix according to claim 50, further comprising a bound troponin C-tagged molecule.

52. A method of producing an affinity matrix according to claim 50, wherein said troponin I is mutated to eliminate internal cysteine residues or to replace internal cysteine residues with other amino acids, wherein said troponin I comprises the sequence CCCSSSSSSSS (SEQ ID NO:3) at the N-terminus or the sequence SSSSSSSSCCC (SEQ ID NO:4) at the C-terminus, and said method comprises reacting said troponin I with a thiol reactive matrix.

53. A method according to claim 52, wherein said internal cysteine residues are replaced with amino acids selected from the group consisting of serine and threonine.

54. A kit comprising an affinity matrix according to claim 42 and instructions for use in a method of purifying a troponin C binding peptide-tagged molecule.

55. A kit according to claim 54, further comprising components for producing a troponin C binding peptide-tagged molecule.

56. A kit comprising an affinity matrix according to claim 42 and instructions for use in a method of purifying a troponin I-tagged molecule.

57. A kit according to claim 56, further comprising components for producing a troponin I-tagged molecule.

58. A kit comprising an affinity matrix according to claim 46 and instructions for use in a method of purifying a troponin C-tagged molecule.

59. A kit according to claim 58, further comprising components for producing a troponin C-tagged molecule.

60. A kit comprising an affinity matrix according to claim 50 and instructions for use in a method of purifying a troponin C-tagged molecule.

61. A kit according to claim 60, further comprising components for producing a troponin C-tagged molecule.

62. A method for detecting a troponin C-tagged molecule, said method comprising contacting a troponin C-tagged molecule with lanthanide ions.

63. A method according to claim 62, wherein said lanthanide ions are selected from the group consisting of lanthanum, terbium, europium, and gadolinium.

64. A method according to claim 62, wherein said troponin C-tagged molecule is detected by luminescence of lanthanide ions bound to the troponin C-tagged molecule.

65. A method according to claim 62, wherein said troponin C-tagged molecule is a troponin C fusion protein.

66. A method according to claim 64, wherein said method is used in an application selected from the group consisting of high throughput screening, study of receptor-ligand interaction, and study of binding kinetics.